PROJECT NUMBER : 1902
PROJECT TITLE : Tobacco Microbiology
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I. TOBACCO MICROBIOLOGY

A. Objective: To develop methods and evaluate the microflora in tobacco materials.

B. Results:

1. 1990 Bright Tobacco Audit

To date, the microbial populations were enumerated from a total of 859 samples of bright tobacco (1,2). The data are being statistically analyzed.

2. Flavor Analyses

A total of four samples of a flavor were submitted for microbial analyses (Lot numbers 319184-07, 08, 09, 13). No microbial growth was detected in any of the samples (3).

3. Bactometer® Study

A completion report was issued (4). Since the instrument was determined to be an inadequate replacement for the plate-count procedure, appropriate disposal plans have been initiated.

4. Cut Filler Analyses - Special Request

A total of eight samples of cut filler were submitted for microbial evaluation (code numbers 1783, 4374, 4370, 2776, 5048, 4376, 3319, 624). The cut filler was initially export material; however, it had been stored in a warehouse since March, 1990. The bacterial and mold numbers were within the expected laboratory limits (5).

5. Microbial Quality Improvement Program (MQIP)

As part of the ongoing MQIP studies, samples were collected and microbially analyzed from a filter making run in the OC Make/Pack facility. The samples included aliquots from the filter tow, hot melt pellets, filter wrap, plasticizer, and wrap glue. No microbial growth was detected from any of the sampled items (6).

6. Effect of Moisture on Visual Mold Growth

A series of desiccators, with different saturated salt solutions to maintain a different relative humidity (RH) in each desiccator, were set up and kept in a walk-in chamber maintained at 37°C. Bright and burley ground strip from the 1989 crop (bright at about 10^4 mold colonies/g, and burley at about 10 mold colonies/g at zero time) were put into the desiccators. At 97% RH, the bright tobacco showed visible mold growth in 4 days, and the burley tobacco showed visible mold growth in 6 days. At 92% RH, bright tobacco showed visible mold in 7 days, and burley tobacco in 12 days. After 5 weeks none of the remaining tobacco samples incubated at 80, 70 and 58% RH showed visible mold growth (7).

C. Plans: (1) Continue statistical analyses and analyze an additional 90 samples of bright tobacco, analyze samples on an "as needed" basis, and continue the study on moisture vs. mold growth.

D. References:

1. Chadick, D. Notebook No. 9044, p. 9.

2. Chadick, D. Notebook No. 8904 pp. 165-174, and 178.

3. Chadick, D. Notebook No. 9044, p. 8.

4. Chadick, D. Completion Report 91-002; 1991 January 25.

5. Chadick, D. Notebook No. 9044, p. 9.

6. Chadick, D. Notebook No. 9044, p. 9.

7. Teng, D. Notebook No. 8788, p. 89.

II. NICOTINE DEGRADATION STUDY

A. Objective: To develop methods for the biodegradation of nicotine.

B. Results: Nicotine bioconversion was studied using different concentrations (MPC-nicotine was diluted with primary influent (PI) at 1:10 and 1:20) of feed and at different rates (1.25-day and 2.5-day retention times) in fermentors containing aeration basin (AB) material. The total suspended solids were maintained above the initial level by a sludge recycle. The nicotine levels in samples of the AB, PI, and MPC liquids along with mixtures of MPC plus PI at different ratios were analyzed at time zero. The amounts of nicotine were also monitored in the AB daily and in the secondary effluent (fermentor overflows) after 1, 5, and 10 days of activity. In addition, the bacterial populations from all the previously mentioned samples were enumerated at time zero and after 1, 2, 5, and 10 days of activity. Except Bu the

MPC-nicotine at a 1:10 dilution and a retention time of 1.25-days, the nicotine concentrations in the other fermentors were below preset concentrations (1).

C. **Plans:** Complete the analyses of TKN, BOD and off-gas.

D. **Reference:**

Tenhet, S. Notebook No. 8281, pp. 196-200.